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SCHISTOSOMIASIS JAPONICA INTERVENTION STUDY ON POYANG LAKE, CHINA: THE SNAIL'S TALE

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ABSTRACT

A U.S. National Institutes of Health-sponsored study was initiated in 1998, ending in 2002, to examine the hypothesis that bovines, buffaloes in particular, were responsible for the persistence of schistosomiasis in human populations living in and around Poyang Lake, Jiangxi Province, P. R. China. The two villages are the focus of this paper, the experimental Jishan Village and the control Hexi Village. The prevalence and intensity of infection of humans and buffaloes were determined. Then, all inhabitants of all the villages were treated with praziquantel, while only the buffaloes of the experimental (intervention) village were treated. Following treatment, rates of re-infection of people in the two villages and buffaloes in the experimental village, as well as the ongoing prevalence of infections in the buffaloes of the control village were monitored annually.

Quantitative collections of snails were made pre- and post-flood for a total of nine seasons to determine the density of snails, density of infected snails, and prevalence of infected snails. Collections were made from ecologically different village zones and from hot spots for persistent snail infections within zones. The hypothesis was that treatments in the experimental village would drive down snail infections to 80% or more below pre-trial levels. This paper makes the case that the intervention did not achieve reducing snail infections village-wide by even 50% or more. The probable reasons for this failure are discussed.

The significant findings of this longitudinal study are several: (1) Foremost, the intervention did not achieve the goal of significantly reducing snail infections in Jishan. (2) To understand the dynamics of *Schistosoma japonicum* transmission throughout large areas, such as the administrative villages of Hexi and Jishan, it is important to partition the area into clearly definable ecological zones. (3) We have found that the greatest risk of infection is in very small areas within zones that we call hot spots. (4) The most important indicator for risk of infection is the density of infected snails, not the prevalence. (5) There are very significantly more infected snails per area in hot spots than in non-hot spots. (6) Density of infected snails is not correlated with density of snails overall. (7) The sampling strategy of zones, squares, and random selection of 20 4 m^2 cells within squares has enabled robust statistical analyses of snail populations that have a strong negative binomial distribution. Sampling twice a year (before the floods and after the floods) for at least 8 or 9 seasons has provided a time series enabling a robust assessment of trends in the experimental and control villages. Paying close attention to environmental factors as they impact results is critical.

Key words: Schistosomiasis, epidemiology, intervention, China, Poyang Lake, *Oncomelania hupensis*, *Schistosoma japonicum*, snail infections, hot spots, disease transmission, ecological partitioning, analysis of variance.

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INTRODUCTION

A U.S. National Institutes of Health-sponsored study was initiated in 1998, ending in 2002, to examine the hypothesis that bovines, buffaloes in particular, were responsible for the persistence of schistosomiasis in human populations living in and around Poyang Lake, Jiangxi Province, P. R. China. The study initially involved four villages, but after two years it narrowed to two administrative villages, a control village and an experimental village. The two villages are the focus of this paper. The baseline study on the demographic factors and human/bovine infections in the villages was published (Guo et al., 2001). The companion baseline study of the distribution, density and prevalence of infections of snails was published (Davis et al., 2002a).

Just prior to initiating the study, the prevalence and intensity of infection of humans and buffaloes were determined. Then, all inhabitants of all the villages were treated with praziquantel while only the buffaloes of the experimental (intervention) village were treated. Following treatment, rates of reinfection of people in the two villages and buffaloes in the experimental village, as well as the ongoing prevalence of infections in the buffaloes of the control village were monitored annually. The results of the intervention for humans and buffaloes are reported elsewhere (Guo et al., 2006).

Each year, snail density and prevalence of infections were determined from different ecological zones of each village in which a number of 10,000 m^2 squares were positioned. All snails were collected from a random selection of frames from 20 cells selected at random from 100 cells in each square. From the collections, the percent area that had snails was calculated along with density of snails and prevalence of infections. Theoretically, the elimination of infections in people and buffaloes in the experimental village should result, within two years, in the elimination of infections in snails, and the life cycle would be broken. One would expect to see a decline of infections in snails so that at the end of this study, there would be no infected snails, or, at the very least, over 80–90% of the pre-study infections (density of infections) would be eliminated. For this to occur in the experimental village, (1) the infections in buffaloes would have to be maintained at zero; (2) there could be no immigration of infected buffaloes; (3) all persons in the experimental village would have to be treated for infections when they were diagnosed to be positive for infections; (4) there could be no immigration of infected snails from outside the village.

The purpose of this paper is to present the snail data collected over nine seasons (from 1988 to 2001) and make the case that the intervention did not achieve reducing snail infections village-wide by even 50% or more. The probable reasons for this failure are discussed.

Background

Poyang Lake, the largest lake in China (area of 4,647 km^2), is a major endemic area for schistosomiasis in China. The lake environ-

TABLE 1. Basic statistics for the two administrative villages. Prevalence data were from the baseline paper for humans and cattle (Guo et al., 2001). Na = No data provided; * = data were not provided by village but an overall mean for the four villages initially studied.

	Hexi Village (Control)	Jishan Village (Experimental)
Number of people	1,043	1,280
Number buffalo	364	665
Grazing area (km^2)	3.69	12.1
Infection prevalence people (1996 data)	Na	17%
Infection prevalence buffalo (1996 data)	Na	20%
Baseline infection prevalence humans	11%	20%
Baseline infection prevalence buffaloes	14%*	14%*

ment is unique. High dikes surround the lake and habitable islands to contain annual flood waters from the spring monsoons. During the annual floods, when the lake basin fills up like a bathtub, all persons and cattle live behind the dykes. With the receding floods, the marshlands once again emerge, and cattle go out on the marshlands to forage. The flooding season extends from April or May to September to October. At the end of the flood, the lake loses as much as 90% of its water. All infections occur within the lake basin in the marshlands; there are no snails behind the dikes. The vast grasslands are difficult to reach and traverse. Travel there is by small boat, water-buffalo carts, and walking.

Population and coordinate data for the villages are found in Davis et al. (2002a). The most relevant summary statistics are given in Table 1. The villages studied are a small subset of the 1,125 endemic villages around Poyang Lake with a total population of 1,750,000 persons and an average infection prevalence of 15.2% (circa 1996). There is about one water buffalo or cow for every 10 persons or 150–200 cattle per village throughout the marshlands. Overall, in hyper endemic villages, 12.9% of the cattle are infected (Davis et al., 2002a).

METHODS

Methods are given in detail in Davis et al. (2002a, b). The baseline data were collected in late October and November of 1998 (post-flood collection and one following the worst flood in China in 40 year) and early May of 1999 (pre-flood collection). Thus, two collections were made each year (spring or pre-annual flood; fall or post annual flood) for a total of nine seasons (ending Nov. 2002). Remote sensing images (Landsat^{tm}) were used to determine the area of the lake, the area of grazing land, and to differentiate snail habitat from grazing area that was not snail habitat (Davis et al., 1999, 2002a, b; Wu et al., 2002). The positions of all sites were recorded using a GPS (Global Positioning System; Garmen handheld GPS) and all data were managed by GIS (Geographic Information Systems).

The villages were divided into clearly observable different ecological zones (three for Jishan, two for Hexi). The zones were defined by factors of elevation, proximity to human habitation where agriculture was one practiced, and degree of prevailing wet-saturated land). Numbers of 10,000 m^2 squares were positioned in the zones, the number employed based on obtaining a representative sample

TABLE 2. The distribution of Hot Spots (*HS*) among the 17 10,000 m^2 squares (identified as A–I) of the five zones of the two administrative villages. The data in brackets are the number of seasons that infected snails were found of the total number of seasons snails were collected, or could be collected, from the square.

Village	Zone	Structure	Square (hot spots)
Hexi	I Shamo	low-flat marshland	A B - *HS* (7 of 9) J
	II Houshan	higher-varied terrain	C D E - *HS* (6 of 9) F - *HS* (5 of 9) G - *HS* (5 of 9) H - *HS* (5 of 7) I - *HS* (6 of 6)
Jishan	I Village	once agricultural land	A - *HS* (7 of 9) D G - *HS* (8 of 8)
	II Southern	low-flat marshland	B C
	III Western	wet-saturated marshland	E - *HS* (5 of 9) F

from each zone. Each square was divided into 100 numbered cells, each 100 m^2. Each season, a random selection of 20 cells was made for each Square. All snails were collected from a 4 m^2 frame placed over the center of the selected cell. The position of each Square and selected cell was recorded each season using the GPS. All snails from a cell were crushed and the number of living snails and number of infected snails scored.

Hot spots (*HS* in Table 2) are those sites where infected snails were found in the majority of seasons, that is, two of three seasons or four of six seasons, and so on. We constantly examined the snail habitat areas for potential sites for hot spots. A special collection of several thousand snails was made from a small area where a hot spot was suspected and the snails crushed to determine if that spot might qualify for a routine collection.

As these snails are notorious for having a negative binomial distribution (i.e., they are highly clumped), we used SAS-8 to perform ANOVAS under a negative binomial model for snail counts. We examined trends through time and looked for significant differences between villages, zones and squares in snail density, infected snail density, and prevalence of infected snails. An offset of log (snail counts) was incorporated into ANOVA models for prevalence of infected snails. In the tables of trend analyses, all data are integrated across seasons. For example, seasons 2–9 (Table 4) does not mean comparing only season 2 data with data from season 9; 2–9 means including data from all seasons from 2–9. Significance is defined as $P \leq 0.05$. In the tables, S = significant difference, NS = not significant, and VS = very (highly) significant ($P \leq 0.01$). Deviation bars in all figures represent 95% confidence limits. Significant difference requires overlap not exceeding 30%, approximately. In some instances when numbers were small, the iterative procedure for fitting the negative binomial model did not converge. These are indicated in results tables by a "no fit" notation.

Mean values discussed in the text are arithmetic means based on the data taken from each cell of a collection. The data plotted in the figures are geometric means, that is, measures of central tendency as we expected changes in the data to occur in a relative fashion. The geometric mean estimates (and their confidence intervals) are obtained using the negative binomial modeling, which takes account of the spatial aggregation. The geometric means are useful summaries for highly skewed data such as found in this study. Thus, there will, in instances, be some small differences in mean values discussed in the text and those found in the figures.

RESULTS

Data from the two villages are compared: (1) for the entire village data, (2) among zones of each village, and (3) among hot spots in each village. The data types for each of the three area sets are snail density, density of infected snails, and prevalence of infected snails. A summary (Appendix 1) provides the snail density data and snail infectivity data in snails per m^2. A summary of all data for each of the five zones of the study is given in Appendix 2. Individual tables and figures involving the data are presented to make graphic presentation more easily displayed. As such, snail density is given as snails per frame (4 m^2), density of infected snails as number per 100 m^2 and prevalence of infected snails as number per 1,000 snails.

Snail Habitat without Snails

Data are presented primarily for the entire area of potential snail habitat. However, there are significant amounts of potential snail habitat that have no snails. There are significant differences among zones in no-snail areas (Fig. 1). Jishan Southern and Hexi Shamo are the most similar in that during both the first half and second half of the seasons studies, less than 4% of the area had no snails. Jishan Village was the most volatile zone with nearly 40% of the area having no snails in the first five seasons, dropping to 21% in the last four seasons. Jishan Western Zone and Hexi Houshan Zone were rather similar, with 25 to 32 % area having no snails in seasons 1–5, dropping to about 5% in seasons 6–9.

In examining the critical hot spots in each village, we also calculate and present the numbers for areas that only had snails given that areas with snails were so patchy in some zones. Data for each hot spot square are plotted as numbers for only those frames that had snails in Figs. 17–22.

Comparing Villages

Snail Density: Changes in the mean number of snails per frame for each season in both villages are shown in Fig. 2. Aside from seasons 2, 3, and 7, the average numbers per

TABLE 3. Trends from seasons 1 to 9 in density of snails per frame (4 m^2) in the two villages: Statistics and probabilities.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		4.75	2.3	7.13	0.0002	VS		4.75	2.3	7.13	0.0002	VS
2 - 9	6.45		2.31	10.56	0.002	VS		1.27	-1.74	4.2	NS	
3 - 9	4.87		0.27	9.67	0.0376	S		6.09	3.21	8.88	<0.0001	VS
4 - 9	5.53		-0.27	11.66	0.062	NS		2.07	-1.5	5.53	NS	
5 - 9		0.7602	-6.144	7.22	NS			4.77	0.53	8.82	0.0279	S
6 - 9	3.907		-4.966	13.61	NS		9.37		3.3	15.8	0.0021	VS
7 - 9	84.82		59.37	114.32	<0.0001	VS	121.69		102.2	143.1	<0.0001	VS
8 - 9	172.68		100.033	271.72	<0.0001	VS	173.68		128.8	227.3	<0.0001	VS

TABLE 4. Comparison of villages for density of infected snails over nine seasons.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		14.87	6.6	22.4	0.0007	VS	9.28		-1.25	20.93	0.0859	NS
2 - 9		20.96	12.82	28.35	<0.0001	VS	12.85		-0.28	27.72	0.05577	NS
3 - 9	0.145		-12.88	15.12	NS		13.63		-1.78	31.46	0.08570	NS
4 - 9		3.3	-15.51	19.05	NS		5.1		-10.77	23.8	NS	
5 - 9		20.39	2.08	35.28	0.0308	S		7.4	-12.84	24.01	NS	
6 - 9		11.83	-18.98	34.66	NS			16.07	-7.83	34.66	NS	
7 - 9	198.6		48.07	502.16	0.0022	VS	54.6		-1.54	142.7	0.05841	NS
8 - 9	242.86		6.98	998.82	0.0381	S		14.29	-89.74	61.28	NS	

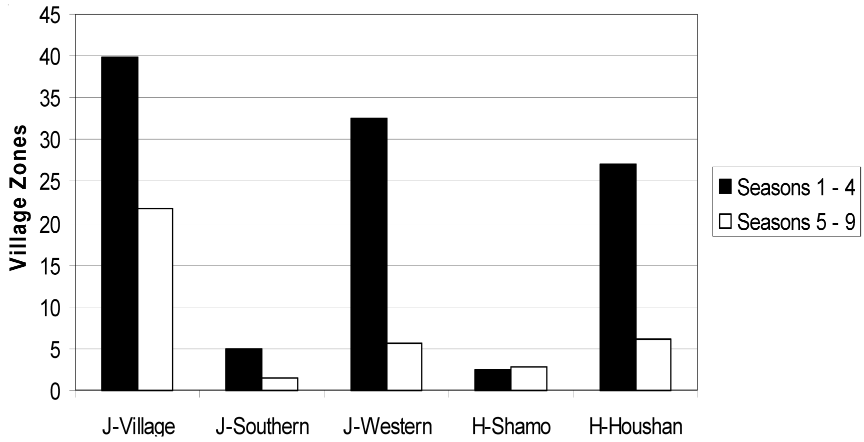

FIG. 1. Percentage of area without snails seasons 1–4 and seasons 5–9.

area each season did not differ significantly between villages. Over the nine seasons, the mean number ± standard deviation of snails per m^2 in Jishan was 10.2 ± 5.3. In Hexi, it was 9.8 ± 5.1 (mean of means for each season). Trends through time are assessed in Table 3. Numbers per m^2 ranged from 4.42–20.00 in Jishan, with the greatest number in season 1. In Hexi, the range was 2.72–15.74 with the greatest number in season 1 (Appendix 1).

Density of Infected Snails: At study end there were no significant differences between villages in densities of infections (comparing seasons 1 and 9) (Fig. 3). Significant points are: (1) There was a big spike of infections in Jishan (intervention village) in season 2 not paralleled in Hexi. (2) Infection levels dropped in seasons 3 and 4 to levels not significantly different from those seen in the baseline data (season 1). (3) There were spikes of infection

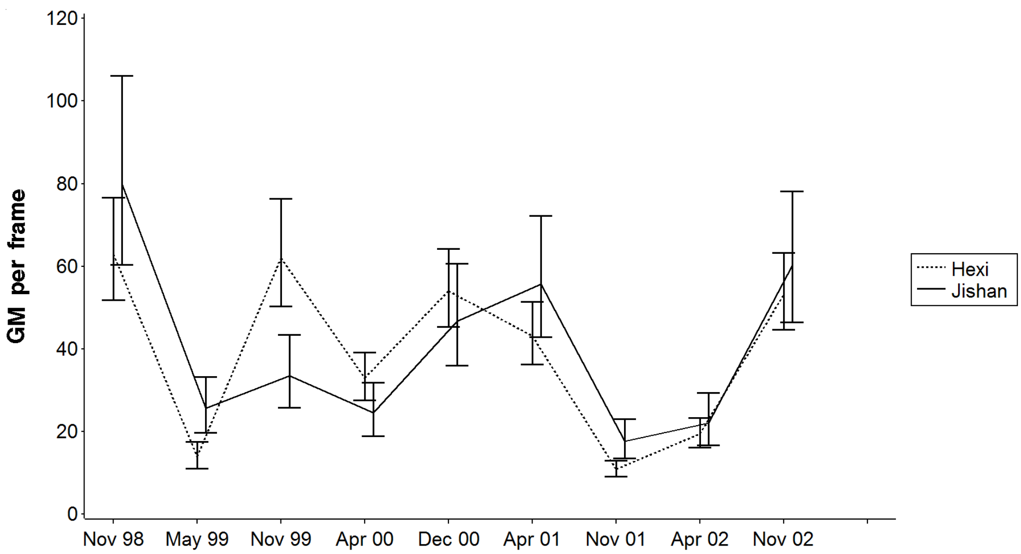

FIG. 2. Comparison of villages for density of snails employing the geometric mean per frame for nine seasons.

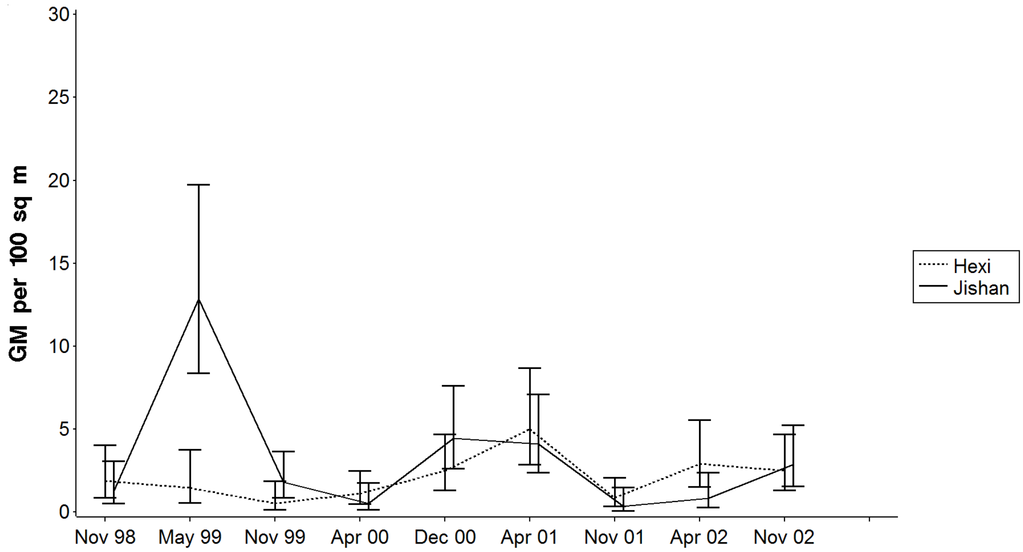

FIG. 3. Comparison of villages for density of infected snails employing the geometric mean per 100 m^2 for nine seasons.

in seasons 5 and 6 (Dec. 2000; Apr. 2001). (4) There were significant drops in infections in season 7 in both villages. (5) As seen in Table 4, there were significant decreases in density of infected snails in Jishan not seen in Hexi comparing seasons 1 to 9 (15%; very significant, P = 0.0007), 2 to 9 (VS), and 5 to 9. However, these are due to the unique and pronounced spike of infections in Jishan in seasons 2 and 5, not to the effects of intervention.

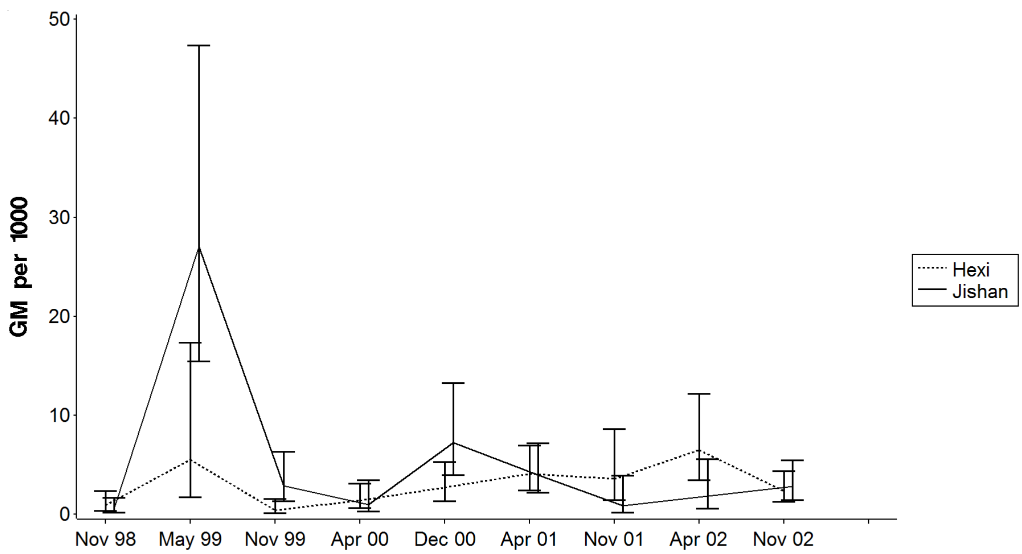

FIG. 4. Comparison of villages for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

Additionally, there was a very significant increase of infection in Jishan from season 7 to 9 (VS) and 8 to 9 (S). Of importance was the occurrence of a severe drought during season 7 and that density of snails and density of infected snails alike dropped considerably in both villages due to inability to collects snails buried in the soil to escape the drought.

In the control village (Hexi), there was no sharp spike of infection in season 2; there was an increase of density of infection in seasons 5 and 6 paralleling that seen in Jishan. There were no significant differences between villages in density of infected snails any season except season 2.

Prevalence of Infected Snails: Villages are compared in prevalence of infected snails (Fig. 4, Table 5). Except for seasons 2 and 3, there were no significant differences. In Jishan, prevalence was lowest in season 1 (0.6/1,000 snails) and highest in season 2 (20/1,000 snails). In Hexi, the lowest was in season 3 (0.3/1,000 snails) and highest in season 8 (6.0/1,000 snails).

In summary, the more important data are the density of infected snails. How many infected snails are found per unit area? Excluding seasons 2 and 7 for Jishan for reasons given above, the greatest was 4.5/100 m^2 in season 5 and least was 0.8/100 m^2 in season 8, but rebounding to 2.9/100 m^2 in the last season. In Hexi the greatest was 5.0/100m^2 in season 6 and least was 0.5/100 m^2 in season 3. Snail density in Jishan was greatest in season 1 (20/m^2) (excluding season 7) and least in season 9 to 5.5/m^2. In Hexi, the greatest was in season 1 (15.7/m^2) and least in season 2 (3.5/m^2).

Comparing Zones

Snail Density (Numbers per Frame): Results for the zones clearly show striking differences among zones in both villages (compare Figs. 5 and 6). In Jishan, zone II, the Southern Zone, had more than double the number of snails in the other two zones; in seasons 1, 5 and 9, more than three times. Numbers of snails in zones I and III were parallel through the seasons excepting the sharp drop in the Western Zone in season 2. As seen later, the sharp upswings in zone II snail density in seasons 1, 5, and 9 had no correspondence to the density of infected snails at those times. In Hexi, zone I (Shamo Grazing land) was similar to zone II (Southern Grazing land) of Jishan in having high density compared to zone II (Houshan). The gap, > 3 to 1 was reduced to no significant difference between zones in seasons 6–9. The average densities per zone in m^2 over all seasons were as follows. High density zones: Jishan-Southern x Hexi-Shamo:

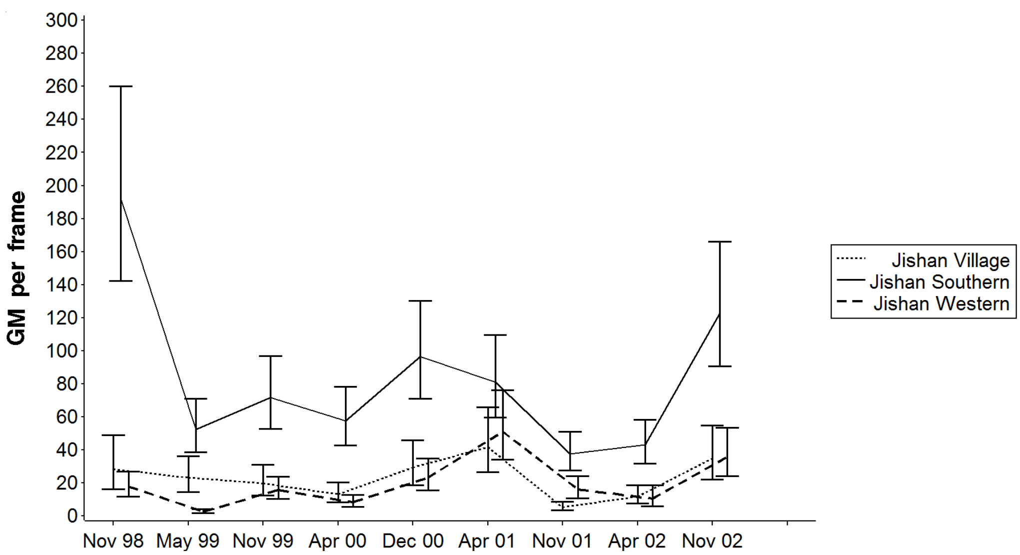

FIG. 5. Comparison of three zones in Jishan Village for density of snails employing the geometric mean per frame for nine seasons.

TABLE 5. Comparison of villages for trends of prevalence of infected snails over nine seasons.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		15.09	5.03	24.1	0.00421	VS	17.32		4.77	31.36	0.00566	VS
2 - 9		23.22	13.91	31.52	<0.0001	VS	13.09		-1.48	29.81	0.08051	NS
3 - 9		5.31	-13.82	21.23	NS		22.08		4.39	42.76	0.01248	S
4 - 9		9.08	-14.58	27.85	NS		11.85		-5.66	32.62	NS	
5 - 9		22.18	-0.55	39.78	0.05508	NS	0.16		-18.7	23.37	NS	
6 - 9		7.896	-33.26	36.34	NS			11	-14.0	30.53	NS	
7 - 9	79.1		-23.58	319.74	NS			26.64	-20.7	55.42	NS	
8 - 9	55.55		-65.86	608.82	NS			63.66	13.62	84.71	0.02195	S

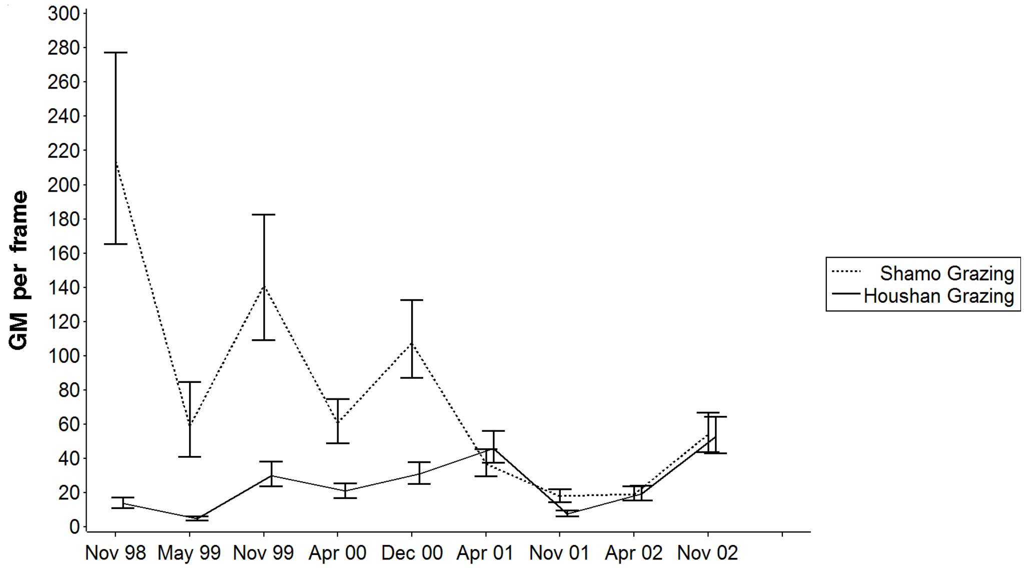

FIG. 6. Comparison of two zones in Hexi Village for density of snails employing the geometric mean per frame for nine seasons.

20.97 ± 12.20; 19.75 ± 16.15. Low density zones: Jishan Village and Western Zones = 5.77 ± 2.94; 4.51 ± 3.44; Hexi Houshan Zone = 6.31 ± 4.08.

Density of Infected Snails (Numbers per 100 m^2*)*: In Jishan (Fig. 7), a sharp jump in density in season 2 was most pronounced in the Village and Southern Zones, with densities

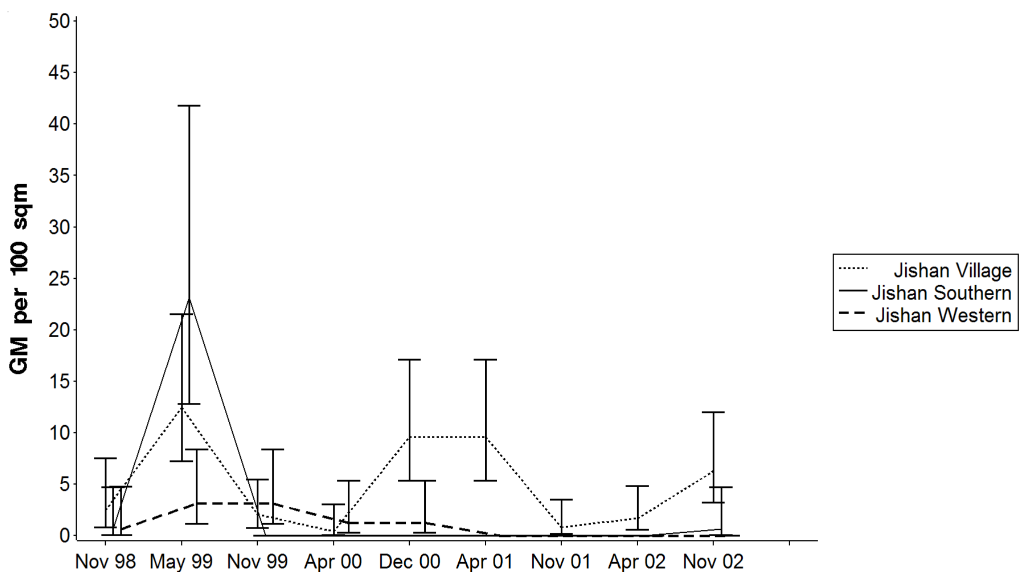

FIG. 7. Comparison of three zones in Jishan Village for density of infected snails employing the geometric mean per 100 m^2 for nine seasons.

TABLE 6. Zones of villages compared for trends in density of infected snails over nine seasons.

	Jishan							Hexi						
Season	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9	Village		5.04	-5.75	14.73	NS		Shamo		29.21	9.046	44.90	0.0069	S
	Southern		55.56	31.18	71.31	0.0003	VS	Houshan	23.41		9.7805	38.74	0.0004	VS
	Western		32.39	7.92	50.36	0.0130	S							
2 - 9	Village		8.96	-1.96	18.72	NS		Shamo		13.095	-31.74	42.67	NS	
	Southern		77.72	53.89	89.24	<0.0001	VS	Houshan	16.52		2.46	32.50	0.0198	S
	Western		47.76	20.56	65.65	0.0024	S							
3 - 9	Village	5.78		-9.00	22.96	NS		Shamo		20.174	-24.50	48.82	NS	
	Southern	NI						Houshan	18.32		1.37	38.10	0.0329	S
	Western		59.39	20.18	79.34	0.0090	S							
4 - 9	Village		2.13	-17.65	18.58	NS		Shamo		30.00	-22.71	60.07	NS	
	Southern	NI						Houshan	9.12		-8.10	29.56	NS	
	Western	no fit												
5 - 9	Village		21.76	3.64	36.47	0.0209	S	Shamo		50.52	-3.47	76.34	0.0616	NS
	Southern	NI						Houshan		2.54	-19.92	20.79	NS	
	Western	no fit												
6 - 9	Village		15.67	-13.81	37.52	NS		Shamo		63.46	-26.7	89.46	NS	
	Southern	NI						Houshan		12.34	-13.23	32.13	NS	
	Western	NI												
7 - 9	Village	no fit						Shamo		no fit				
	Southern	NI						Houshan	62.93		2.7	158.50	0.0382	S
	Western	NI												
8 - 9	Village	no fit						Shamo	NI					
	Southern							Houshan		14.29	-90.54	61.44	NS	
	Western													

reaching 12.5/100 m^2 (0.125/m^2) and 23.1/100 m^2 (0.231/m^2) respectively. Thereafter, densities declined dramatically in season 3 in the Southern Zone and were zero until the last season when infections re-emerged. Densities decline in the Western Zone from season 3 to disappear in season 6, thereafter not to reappear. By contrast, there were no significant changes in density in the Village Zone comparing seasons 1, 3, 4, but a significant increase from season 4 to seasons 5 and 6, jumping from 0.425/100 m^2 to 9.57/100 m^2. The levels in seasons 7 and 8 were not significantly different from those seen in seasons 1, 3, and 4. In season 9 there was a rise to a mean of 6.25/100m^2 (contrasted with 2.5/100 m^2 in season 1).

Considering significant changes in the Village Zone (Table 6), the only significant trend following the spike in season 2, was from season 5 to 9 ($P = 0.021$) following the elevated spike of infection in season 5 and maintained in season 6. Considering the other two zones, from season 3 onward, there were obviously significant decreases in the other two zones as numbers of infected snails went to zero in season three in the Southern Zone; in season 6 in the Western Zone.

Densities of infected snails in Hexi Village (two zones) are shown in Fig. 8. With the exceptions of seasons 1, 2, and 9, there were no significant differences between the zones. However, the average densities were generally higher in the Houshan Zone. The mean of means over nine seasons in the Shamo Zone was 1.37 ± 2.22 infected snails per 100 m^2) contrasted with 2.44 ± 2.02 in the Houshan Zone. Both the Hexi Zones are lower than the 3.79 ± 3.68 found in the Jishan Village Zone.

There was no spike in numbers of infected snails in the second season in Hexi. There was a significant decline in density of infected snails in Shamo from season one to nine (Table 6). Thereafter, there were no significant changes in density until the last season when numbers of infected snails dropped to zero. In the Houshan grazing range, there was a contrasting result. There were significant increases in density of infected snails comparing seasons, 1, 2 and 3 to season 9; and season 7 to 9.

Prevalence of Infected Snails: Prevalence's do not precisely mimic trends of density (contrast Figs. 7 and 9 for Jishan Village). As with density data, focus is on the Village Zone. Isolating the effect of the spike in season 2, there was no significant difference among seasons except for that involving season 5 in which there was a pronounced increase in prevalence from season 4. The only significant trend decrease in prevalence was from season 5 to season 9 (21.37%; $P = 0.03$; Table 7).

In Hexi, there was a spike in prevalence of infected snails in season 2 in the Houshan Zone (Fig. 10). While it would appear that there was a slow increase in prevalence in the Houshan

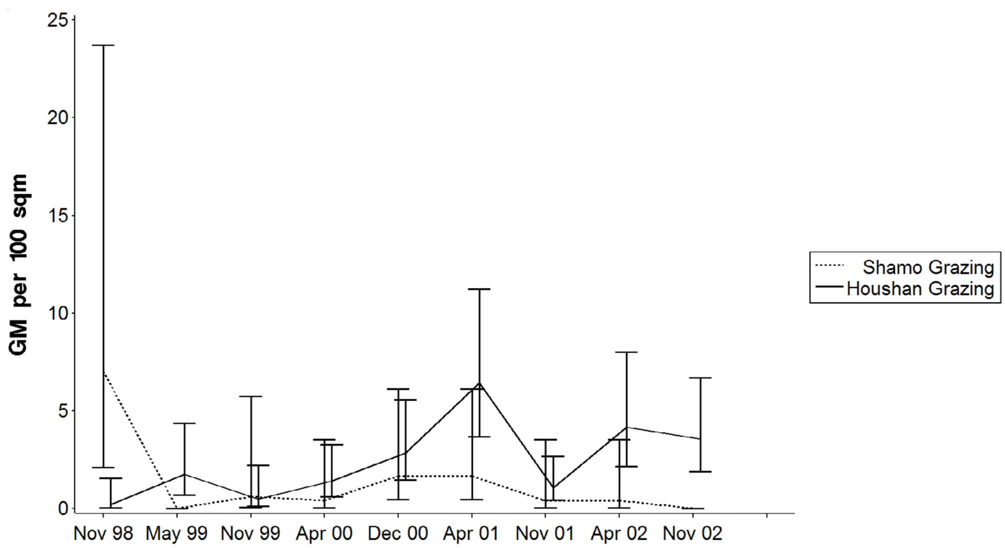

FIG. 8. Comparison of two zones in Hexi Village for density of infected snails employing the geometric mean per 100 m^2 for nine seasons.

TABLE 7. Comparing zones of the villages for trends in prevalence of infected snails over nine seasons.

	Jishan							Hexi						
Season	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9	Village		4.66	-6.82	14.91	NS		Shamo		5.07	-19.47	24.566	NS	
	Southern		56.51	31.00	72.59	0.0004	VS	Houshan	12.35		-0.83	27.274	0.067	NS
	Western		54.20	26.42	71.49	0.0013	VS							
2 - 9	Village		10.09	-1.25	20.16	0.0793	NS	Shamo	15.28		-22.87	72.291	NS	
	Southern		84.38	66.34	92.75	<0.0001	VS	Houshan	6.33		-7.268	21.931	NS	
	Western		70.03	50.40	81.89	<0.0001								
3 - 9	Village	2.14		-13.37	20.41	NS		Shamo	15.28		-22.87	72.291	NS	
	Southern	no fit						Houshan	6.33		-7.268	21.931	NS	
	Western		67.86	36.01	83.86	0.0012	S							
4 - 9	Village		4.90	-15.49	21.69	NS		Shamo	9.97		-27.47	66.715	NS	
	Southern	no fit						Houshan	15.48		-1.505	35.248	0.076	NS
	Western		88.89	24.72	98.36	0.02	S							
5 - 9	Village		21.37	1.91	36.96	0.03	S	Shamo		4.62	-58.19	42.488	NS	
	Southern		no fit					Houshan	7.23		-9.794	27.47	NS	
	Western			99.96	-4.87	100.00	NS							
6 - 9	Village			16.73	-13.24	38.77	NS	Shamo		22.39	-46.73	58.945	NS	
	Southern		no fit					Houshan		1.51	-21.65	20.266	NS	
	Western		no fit											
7 - 9	Village		18.10		-45.57	156.23	NS	Shamo		74.58	-116.1	97.01	NS	
	Southern		no fit					Houshan		29.94	-15.91	57.655	NS	
	Western													
8 - 9	Village		35.07		-62.91	391.91	NS	Shamo	no fit					
	Southern		no fit					Houshan		62.32	10.405	84.154	0.0272	S

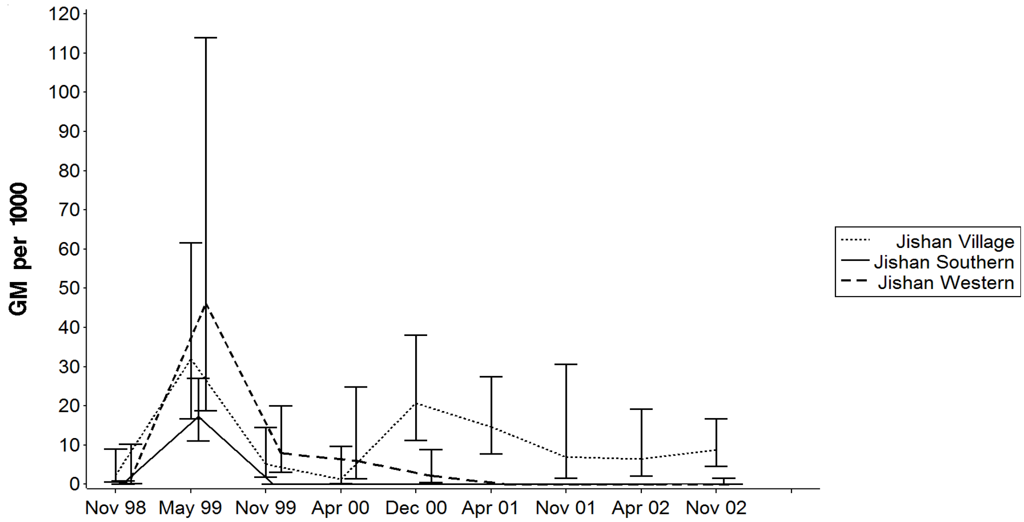

FIG. 9. Comparison of three zones in Jishan Village for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

Zone from season 3 to 9, there was no significant trend, except for a significant decrease from season 8 to 9 (Table 7, 62%; P = 0.027).

Hot Spots

The persistence of infected snails within a 10,000 m^2 area has profound implications for the epidemiology of schistosomiasis (Table 2). In Jishan, two of three such areas were hot spots in the Village Zone closest to the main cluster of human habitation and frequently visited by villagers and buffaloes. The Western Zone, far removed from the natural villages, had one hot spot that existed only 5 of 9 seasons (disappearing by the 6th season). By contrast, Hexi had more hot spots with 5 of 7 squares in the Houshan Zone and one of three in Shamo.

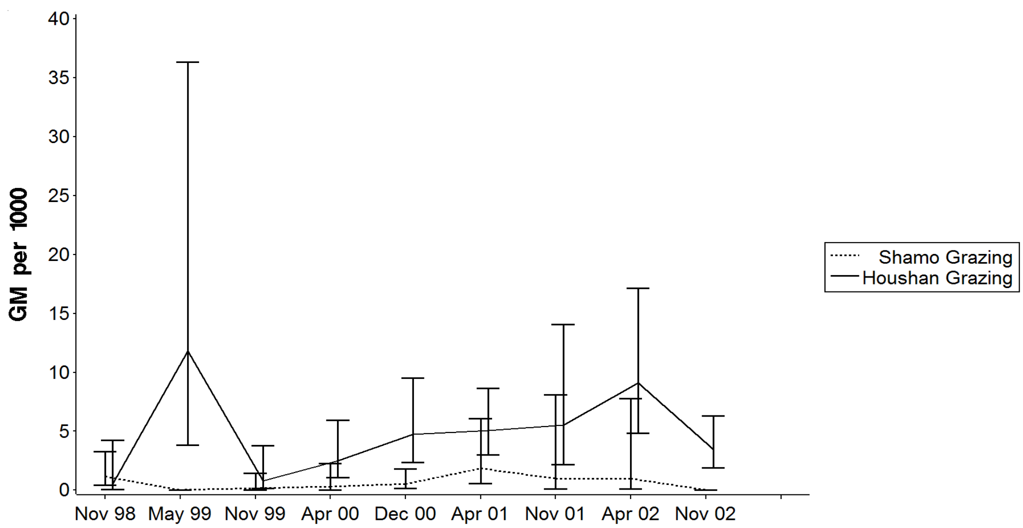

FIG. 10. Comparison of two zones in Hexi Village for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

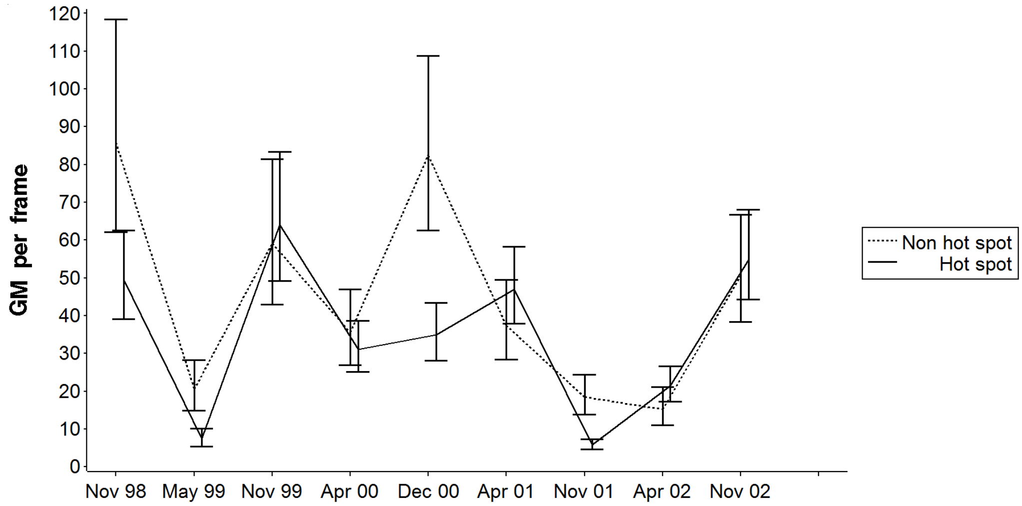

FIG. 11. Comparison of snail densities in Jishan Village for hot spots vs. non hot spots employing geometric mean per frame.

Hot Spots vs. Non Hot Spots Villages: The two villages differed in density of snail hot spots vs. non hot spots (Figs. 11, 12). In Jishan, there were significantly more snails per area in non hot spots than in hot spots (average of averages of snails per frame over the nine seasons of 49.3 ± 25.4/frame vs. 28.2 ± 16.6 per frame). Of note was the surge of snails per frame in seasons 5 and 6. In Hexi, there was no significant difference except in season 5. The average of averages of snails per frame over the nine seasons was 38.5 ± 21.9 in hot spots vs. 45.7 ± 33.7 in non hot spots.

Density of Infected Snails in Hot Spots vs. Non Hot Spots: Compared to density of snails, the situation of density of infected snails is quite different in that there were significantly more

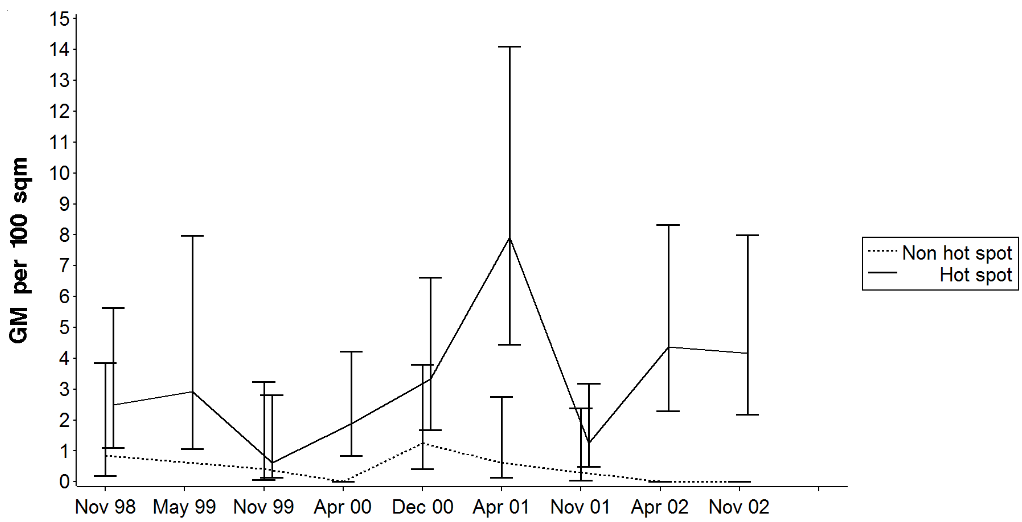

FIG. 12. Comparison of snail densities in Hexi Village for hot spots vs. non hot spots employing geometric mean per frame for nine seasons.

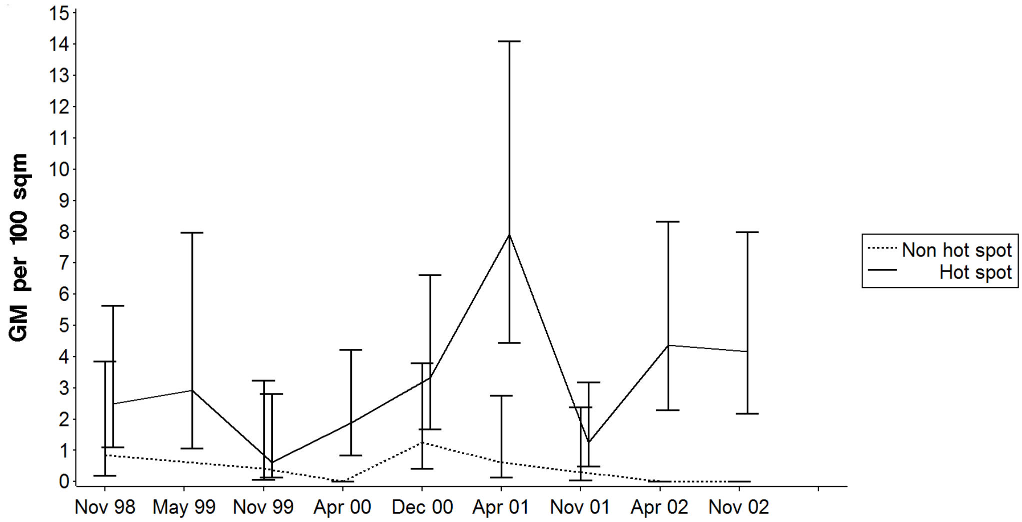

FIG. 13. Comparison of density of infected snails in Hexi Village for hot spots vs. non hot spots employing geometric mean per 100 m^2 for nine seasons.

infected snails per area in hot spots than in non hot spots. The mean of means over nine seasons was 3.5 infected snails per 100 $m^2 \pm 2.0$ in hot spots vs. 0.84 ± 0.91 in non hot spots in Hexi (Fig. 13). For Jishan, the comparative data were 5.3 ± 4.7 in hot spots; 1.7 ± 3.7 in non hot spots. The high standard deviations reflect the sharp spike of infected snails in season 2 (Fig. 14).

Prevalence of Infected Snails in Hot Spots vs. Non Hot Spots: The difference in number of snails per 1,000 snails found in hot spots

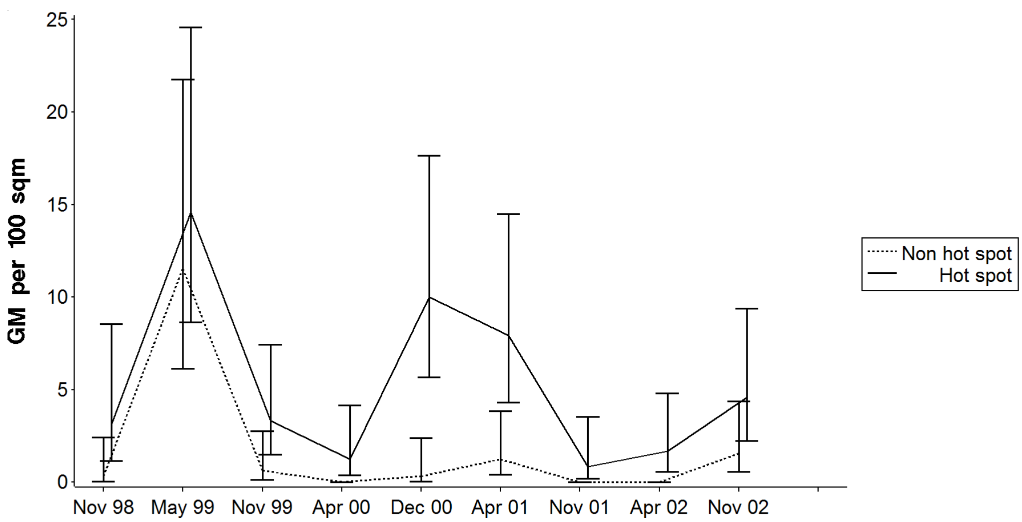

FIG. 14. Comparison of density of infected snails in Jishan Village for hot spots vs. non hot spots employing geometric mean per 100 m^2 for nine seasons.

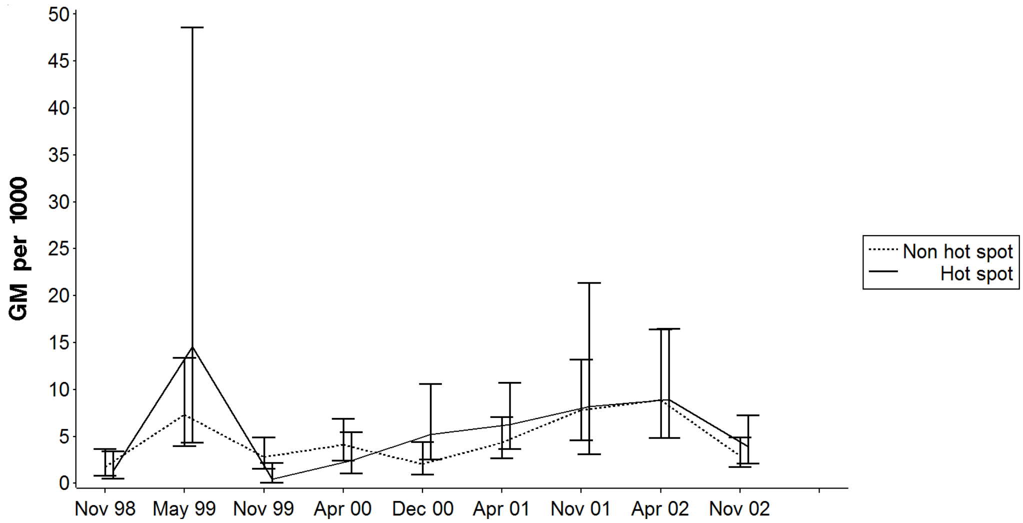

FIG. 15. Comparison of prevalence of infected snails in Hexi Village for hot spots vs. non hot spots employing geometric mean per 1,000 snails for nine seasons.

vs. non hot spots was much more pronounced than the density of infected snails. In Hexi (Fig. 15), the mean of means over nine seasons in non hot spots was 0.9 ± 0.97, whereas in hot spots it was 5.6 ± 4.88. The trend over the nine seasons was a sharp decease from season 2 to 3, then a regular increase from seasons 3 to 7 declining slightly to season 9. In Jishan (Fig. 16), the mean of means over nine seasons in non hot spots was 2.2 ± 0.3. Excluding the anomalous spike in season 2, the result was 0.3 ± 0.38. The prevalence was

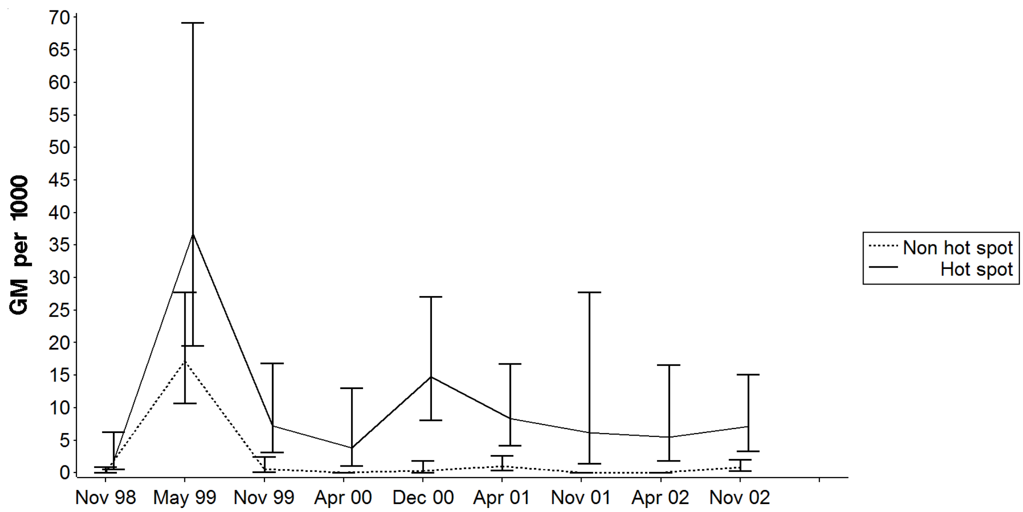

FIG. 16. Comparison of prevalence of infected snails in Jishan Village for hot spots vs. non hot spots employing geometric mean per 1,000 snails for nine seasons.

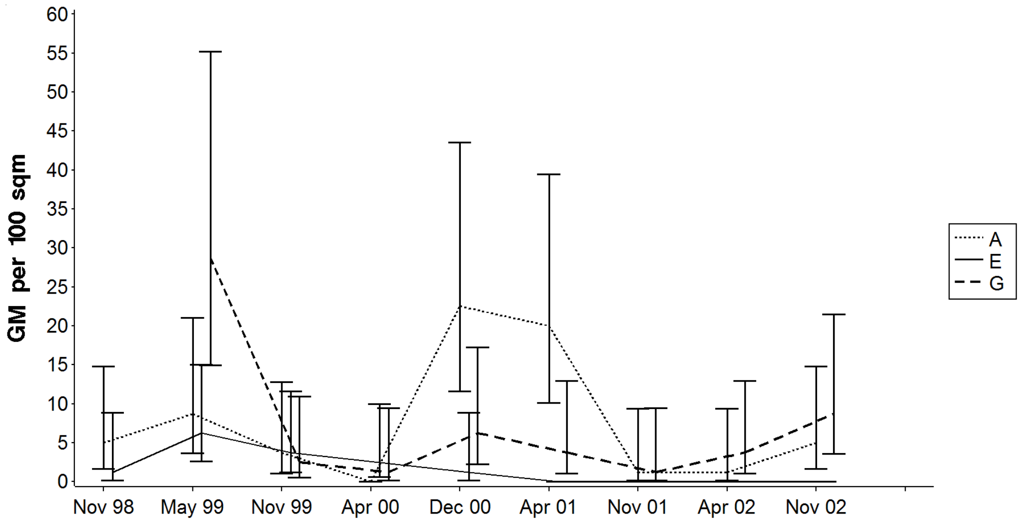

FIG. 17. Densities of infected snails in hot spots in Jishan Village: Village Zone (squares A, G) and Western Zone (square E) employing geometric mean of snails per 100 m^2.

considerably higher in hot spots. The mean of means over nine seasons was 7.5 ± 6.32. Excluding the spike in season 2, the result was 5.5 ± 2.2. As seen in Fig. 16, from season 3 onward the geometric mean of number of infected snails is not significantly different from 9/1,000. There was no net decrease over time.

Hot Spots in Zones – Density of Infected Snails

Jishan Village: The highest risks for gaining infections were in hot spots as seen by comparing the high risk Village and Western Zones with the low risk Southern Zone. There were two squares in the Southern Zone, but neither

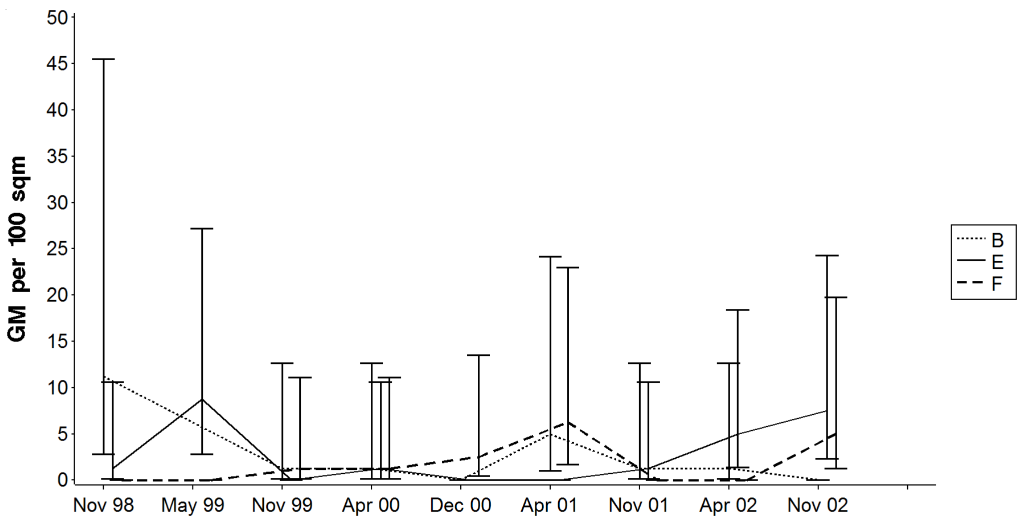

FIG. 18. Densities of infected snails in hot spots in Hexi Village: Shamo (square B) and Houshan (squares E, F) Zones employing the geometric mean per 100 m^2.

qualified as hot spots. Square C had 1 and 30 infected snails in seasons 1 and 2 respectively (= 1.47 and 19.44 infected snails per 100 m^2). Thereafter, no infected snails were collected. In the last season, after having no infected snails, one was found in square B (= 1.25 per m^2).

The most continuous substantial risks for infections were in the Village (sites A, G) and Western Zones (site E), but of these the zone closest to human and buffalo activity, the Village Zone, had the most severe continuous risks (Fig. 17). The density of infected snails in the Western Zone, after a marked increase in density and prevalence in season two gradually dropped to zero in the 6th season. However, in the Village Zone, the two hot spots had very different patterns of snail infections. In square A (Mexi Lake), the density of infections went from 11.7/100 m^2 in season 2 to zero in season 4, only to spike up to 25 and 22.2/100 m^2 in seasons 5 and 6 before dropping to levels of 5/100 m^2 or less in the last three seasons. In square G, very close to village houses, the pattern was quite different. In season 1 the site had not yet been discovered. In season 2, there was an extraordinary spike of infection (35.9/100 m^2). Density dropped in seasons 3 and 4 to levels not significantly different from levels seen in the other sites. Densities increased to over 5/100 m^2 in seasons 5 and 6, rising to 9.2/100 m^2 at the end of the study (the drop in season 7 seen in all sites was due to severe drought driving snails underground and thus uncollectible). For the Village Zone overall, statistically, the trends for these data are not significant comparing seasons 1, 2, 3, 4 to 9. There was a significant drop from season 5 to 9 attributed to the high spike in season 5 in square A (22% decrease, P = 0.021).The decrease from season 6 to 9 was not significant.

Hexi Village: There were two very different ecological zones. The east-of-plateau Shamo Zone equaled in every way the Southern Zone of Jishan in topography, high density of snails, density and abundance of grass and abundance of small ponds and pools in the "dry" season. Of three sites in Shamo, one hot spot (B) was found, a site closest to village daily activity. Due to flooding, it could not be collected in the second season; it had no infected snails in seasons 5 and 9. It had 11.25 infected snails/100 m^2 in season one and thereafter never exceeded 1.39/100 m^2. Analysis of trends over the nine seasons showed no significant differences (Fig. 18). Overall, in spite of this large area (Shamo) having high snail density, the density of infected snails was low to zero.

Houshan Zone was much more complex (Figs. 18, 19). Due to its size and irregular topography, six squares were originally chosen

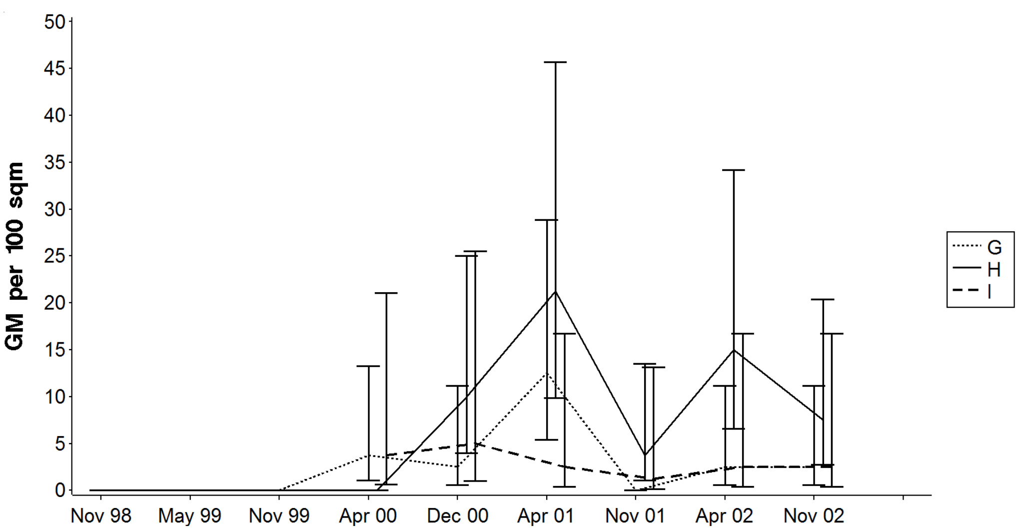

FIG. 19. Densities of infected snails in hot spots in Hexi Village: Houshan Zone (squares G, H, I) employing the geometric mean per 100 m^2.

with a 7th added in the 4th season, because a potential hot spot was discovered using the investigative technique of the "special collection" (see Methods). Of the seven sites (squares), five were hot spots. None went to zero infected snails for more than 3 or 4 seasons. Site G was flooded out one season and could not be collected; it was inadvertently not collected in the second season. Each hot spot was unique in pattern of density of infected snails. Only E had a significant spike of infection in the second season but not nearly as high as the unique spike in Jishan G (13.46/100 m^2 vs. 35.94 – three times higher). Four hot spots had elevated levels of infection in season 5 and 6 paralleling the increases seen in those seasons in Jishan A and G, but especially G.

As in Jishan, density of infected snails was low in season 7 due to the severe drought, and as in Jishan, increased in seasons 8 in E, G, H , and I. There was a significant increase at E in the last season. Taking Houshan hot spots as a whole, the trends of each season to season 9 were not significant except from season 8 to 9, when there was a significant 62.3% increase ($P = 0.027$). The parallel trend in Jishan (season 8 to 9) of 35% increase was not significant.

Comparing Hexi and Jishan: In Jishan, density in Village Zone site A started at 7.14 and was ≥ 5.0 in seasons 5, 6, and 9. At site G, the same held true in seasons 5, 6, 8 and 9. In the control village of Hexi, considering the critical seasons 5, 6, 8, and 9, and to make a comparison with the Jishan data above, hot spot B in Shamo had densities above the mean line of 3.54 infected snails/100 m^2 only in season 6, thereafter dropping below 1.50 and reaching zero in season 9. In Houshan sites F, G, and I, densities above 5.0/100 m^2 were attained in seasons 5 or 6 only to drop below 3.54 thereafter. There were increases above 3.54 in seasons 8 and 9 at sites E and H. In summary, in Hexi, there were only significant gains in density in seasons 8 and or 9 in just two hot spots, E and H.

Hot Spots in Zones- Prevalence of Infected Snails

Jishan: Prevalence data for hot spots are given in Fig. 20. As with density of infected snails, prevalence of infected snails had different dynamics in different hot spots. In the dominant at-risk Village Zone, there were two very different dynamics. At site A, season four had no infected snails and a spike to > 15 in season 5. Thereafter, the prevalence dropped to a level between two and four, not significantly different from what was found in season 1. In stark contrast, at site G, from season four onward the overall trend was from four infected snails per 1,000 to 40 in season 6,

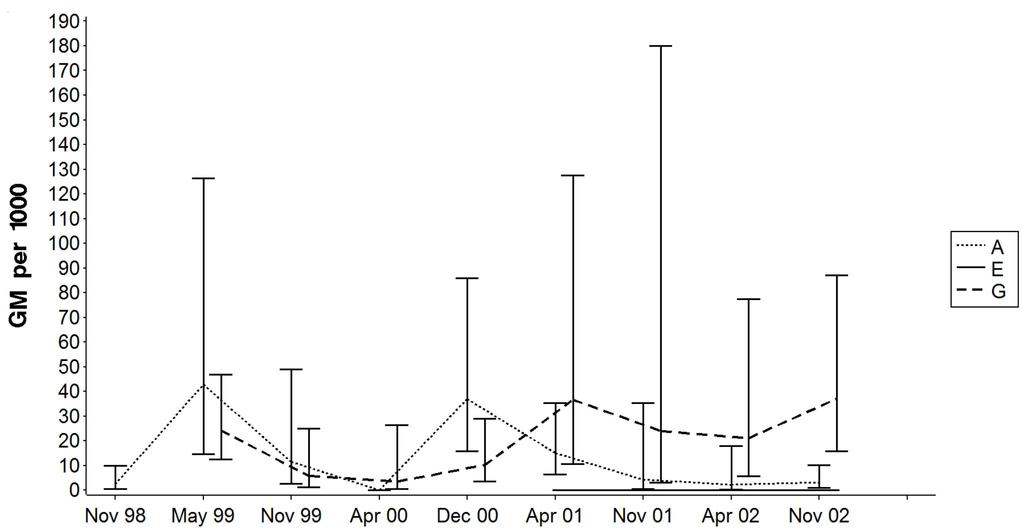

FIG. 20. Prevalence of infected snails in Jishan Village hot spots: Village Zone (squares A, G) and Western Zone (square E) employing the geometric mean per 1,000 snails.

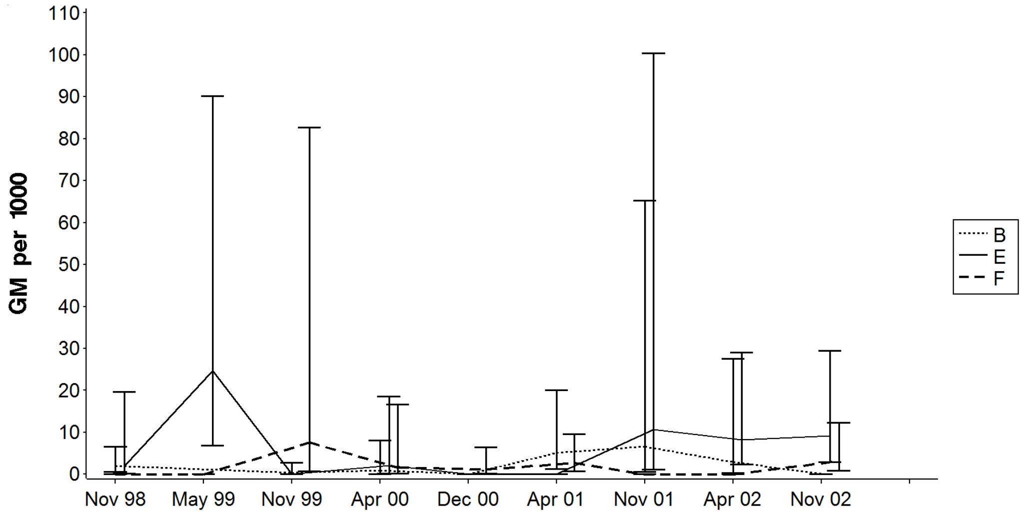

FIG. 21. Prevalence of infected snails in Hexi Village hot spots: Shamo Zone (square B) and Houshan Zone (squares E, F) employing the geometric mean per 1,000 snails.

decrease to 30 in season 8 and then a spike to 37 in season 9.

Hexi: Prevalence data for hot spots are given in Figs. 21 and 22. As in Jishan, there were very different dynamics among hot spots. Only Houshan E had a spike in prevalence of infected snails in season 2. From seasons 3 onward (post season 2 spike), there was a trend for significant increase by season nine only in Houshan sites E, and this a modest increase (< 10/1,000). Dramatic changes in prevalence were found at three Houshan sites: G, H, and I. From prevalence's ≥ 14/1,000, they all dropped to 6/1,000 or less in season 9. The only commonality among hot spots is a significant increase in prevalence in seasons 5 or 6, the same trend seen in Jishan.

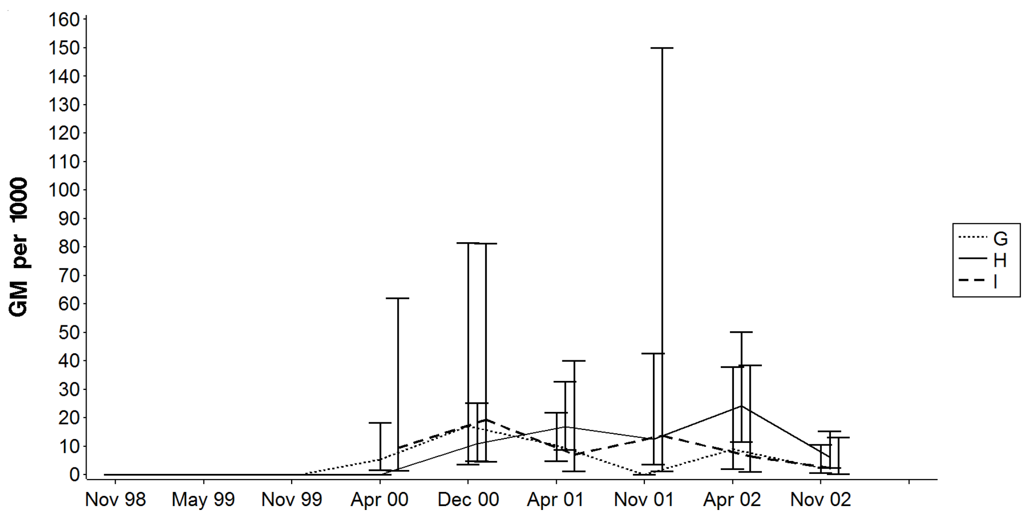

FIG. 22. Prevalence of infected snails in Hexi Village hot spots: Houshan Zone (squares G, H, I) employing the geometric mean per 1,000 snails.

DISCUSSION

The significant findings of this longitudinal study are several:(1) Foremost, the intervention did not achieve the goal of significantly reducing snail infections in Jishan. (2) To understand the dynamics of *Schistosoma japonicum* transmission throughout large areas such as the administrative villages of Hexi and Jishan, it is important to partition the area into clearly definable ecological zones. (3) We have found that the greatest risk of infection is in very small areas within zones that we call hot spots. (4) The most important indicator for risk of infection is the density of infected snails, not the prevalence. (5) There are very significantly more infected snails per area in hot spots than in non hot spots. (6) Density of infected snails is not correlated with density of snails overall. (7) The sampling strategy of zones, squares, and random selection of 20 4 m^2 cells with squares has enabled robust statistical analyses of snail populations that have a strong negative binomial distribution. Sampling twice a year (before the floods and after the floods) for at least 8 or 9 seasons has provided a time series enabling a robust assessment of trends in the experimental and control villages. Paying close attention to environmental factors as they impact results is critical. These findings will not necessarily be discussed in the order given above.

The Intervention

Theoretically, the treatment of people and water buffaloes in Jishan to rid them of infection should end the input of miricidia to snails. All infected snails could be expected to die within 12 to 18 months, and there would be no further source of infection. Over the nine seasons, with all sources of infections removed, schistosomiasis would be eliminated locally. Infected snails are the key! At the very least, one would expect a continuous decrease in density of infected snails in the experimental village compared to the control village. One should have at least an 80–90% reduction of infected snails. This did not occur. A quick look at Fig. 3 says it all at the village level. From season 3 onward the two villages did not differ significantly in density of infected snails (excepting season 8). There was no discernable trend towards < 0.5 infected snails per 100 m^2. There should have been no increases in infected snail density in seasons 5 and 6, more than half way through the experiment.

The probable reasons for the failure of the intervention are given at the end of the discussion after other factors are discussed.

Ecology, Environmental Factors, and Results

Snails must be sampled twice a year at the very least. The reasons for this are that these marshland ecosystems are dynamic with regard to variances in environmental factors. The vast marshland systems of Jiangxi and Hunan (Poyang and Dong Ting Lakes) are subjected to severe annual flooding. The floods coincide with the spring-summer monsoons. The timing and intensity of the monsoons vary. These combined with considerable season variance of temperature have a profound effect on snail populations.

Likewise, high temperatures and pronounced drought in summer severely affects snail populations. If the lakes flood early and the temperature is low, as in the severe floods of 1998, there will be one set of negative factors for snail survival and reproduction. If flood waters do not recede by late September, there will be other consequences. Late minimal flooding and early recession of floods (optimal conditions for snails) yields yet other results. All these factors relate to disease transmission. One cannot predict the timing of adverse affects on snail populations at either end of what is considered the “usual” flooding period.

In this study, it is clear that there is no clear seasonal bias on results. For example, one does not obtain consistently high numbers in the fall and low numbers in the spring. As the sampling technique does not enable picking up immature snails, the adults are collected in both seasons. Of note is that “old” adults are collected in both seasons, that is, a significant number of “older” adults survive the floods. So again, it is important to sample both sides of the flood period.

The extreme negative impact of the 1988 floods on snail populations, usually heavily infected, along the Gan River in Xinhua Village of Poyang Lake was documented (Davis et al., 2002a). The village flood plain was swept clear of snails by the extreme floods. In November 1988, about 90% of the entire area had no snails. Gradually snails re-colonized the area so that by December 2000 < 5% of the area had no snails. Snails per meter square in the one Xinhua hot spot increased from < 20 per frame in March 1999 to 72 by December 2000.

Given the example of Xinhua, it would appear that there was no adverse affect of flood-

ing in the Jishan-Hexi Village marshlands. None of the Hexi-Jishan marshlands were swept clear of snails. In November 1988, there were, on average, > 65 snails per frame in both villages (no significant difference), and at the end of the experiment there were > 50 snails per frame (no significant difference between villages). Only in season two (May 1999) was there a significant drop in density in both villages to 15–20 per frame (Jishan had significantly more), levels similar to those seen in season 8. Accordingly, one cannot attribute the infection data to flood related decrease in snail density at the beginning of the experiment.

There was apparently a flood-related impact on the percentage of area that had no snails as seen in Fig. 1. Three zones had > 25% area with no snails from seasons 1–4 that decreased to 6–21% in seasons 5–9. The zones with the highest snail densities (Jishan-Southern and Hexi-Shamo) did not have such large percentages of land without snails throughout the experiment. As more area obtained snails in the zones indicated, there is no evidence that this impacted overall snail density or density of infected snails. The snails simply expanded into previously vacated habitat.

Anomalous Results

There are two results that are considered anomalous in this experiment. One can be explained on the basis of ecological observations made during each sampling period. There is, as yet, no explanation for the other. The explainable anomaly is the season 7 low numbers in all data. These low numbers in Jishan are not the result of the intervention. The same low numbers were found in Hexi. There was a sever drought on the marshlands in the late summer and fall of 2001. With the prolonged absence of water the snails go underground and aestivate. Sampling during this period yielded numbers that considerably underestimates the actual number of snails present. To illustrate this point, one of us (GMD) went to collect snails from Lao Zhou Island, Anhui, in June some years ago. The island was available then for the very reason that the monsoons were delayed and there had been a summer drought. During the monsoons the island flood plains are under water. The morning collection of *Oncomelania* by some 15 persons yielded a negligible number of snails (< 2 snails/m^2). During the end of the collection period, it began to rain. We left the area and returned three hours later, during which time the rain was continuous and heavy. We again collected the same area and obtained a high density yield (> 28 snails/m sq). With the available moisture soaking the ground the snails came to the surface to resume activities associated with an optimal habitat. The sampling problem seen above is one encountered by all standard collecting procedures used. The caution is to attempt to collect during periods of normal soil moisture associated with active snail behavior.

The other anomalous result was the exceptional spike of infected snails in season two in Jishan. The 19.5 infected snails per 100 m^2 for Jishan Village as a whole is far above the data recorded for the lake region. To our knowledge, it is a unique occurrence. The historical record for this quadrant of the lake averages 2 per 100 m^2 (Anonymous, 1985; Davis et al., 2002). The spike is primarily a phenomenon of hot spot G in Jishan where the number > 35/100 m^2. The only plausible explanation is that one or more heavily infected buffaloes defecated directly on this site several times over a period of time infecting numerous snails in the restricted area. It occurs when the density of snails actually had a significant decrease.

If one ignored these anomalies, and used ANOVAS to only examine trends from season one to season nine, one would come to an erroneous conclusion. For example, as there is a significant decrease in infected snails from season one to season 9 (15%; P = 0.00007) one might be tempted to say that the intervention was working. This clearly was not the real situation as discussed above.

Zones, Snail Density, Snail Infections, and Hot Spots

Remote sensing using Landsat TM images has enabled classification of the these marshlands into three general types: (1) mud flats grading into areas supporting some grass; (2) cattle grazing range that grades from water-covered grass extending throughout the marshlands that support grasses of all types, including higher dryer areas that can still support grass; and (3) snail habitat where the land within the larger cattle grazing range is both dry enough and moist enough to support snails (Davis et al., 2002b).

In this study, areas that were capable of supporting snails were divided into ecological zones. The results show the value of partitioning the villages into ecologically discernable

zones. Two zones, Jishan-Southern and Hexi-Shamao, had the most ideal snail habitat over a vast area and supported the greatest density of snails, generally over 60 snails per frame (15/m^2; medium to high density, Table 2, Davis et al., 2002a). In Jishan, the Village Zone was less than optimal snail habitat due to the fact that in recent years rising lake levels were forcing the conversion of that zone from farming fields to marshland. The zone did not have the vast level land at an elevation above mean low water supporting lush growths of thick, tall grasses that provide the shade and humidity most suitable for *Oncomelania.* The Western Zone of Jishan had large areas much too wet for optimal *Oncomelania* habitat. In this zone grasses were frequently partially to wholly covered by water for prolonged periods of time. In these two less than optimal zones snail density was generally < 35 snails/frame (8.5/m^2; low density). For reasons we cannot explain, in the last three seasons (excluding the dry seventh season), the snail density in Shamo dropped to levels insignificantly different from those on the Houshan Zone.

The Houshan-Zone of Hexi was one dissected by ingressions from the lake and large areas sloping up to elevations marginal for snails. Overall, the places most suitable for snails were restricted into narrow strips of land supporting rich stands of grass bordering the ingressions from the lake. Over the first five seasons the density was low. In the remaining seasons (excluding the dry period of season 7) the density increased to about 45/frame (11/m^2; medium density).

It is counter-intuitive that the snail dense zones do not have the highest density of infected snails, but that is the situation. This was noted before (Davis et al., 2002b) for Hexi and Jishan. The correlation between snail density and prevalence of infection for all sites in Hexi, after seven seasons, was r = -0.189; in Jishan r = -0.210. Considering all nine seasons for both villages, the correlation between snail density and density of infected snails was r = -0.134. Highest density areas for snails such as Shamo in Hexi and the Southern Zone in Jishan have the lowest density of infected snails. In Hexi- Shamo, the mean of means for snails/frame was 79; infected snails per 100 m^2 were 1.4. The contrast with the Houshan Zone was 22 snails per frame and 2.3 infected snails per 100 m^2. In Jishan, the mean of means for snails/frame in the Village Zone was 23; infected snails per 100 m^2, 3.8. The contrast with the Southern Zone was 84 mean number of snails/frame. Infections dropped to zero after season two. In seasons 1 and 9 (excluding the anomalous spike in season two) the infected snails/100 m^2 averaged below 2.1.

Hot spots account for the high density of infected snails in areas otherwise with low density snails. It is informative that in snail rich Shamo, only one of six hot spots was maintained and this a rather weak one. As shown, densities of infected snails in hot spots were considerably higher than densities in non hot spots. In Hexi, infected snail densities in hot spots averaged 3.5 ± 4.5/100 m^2; in Jishan, excluding the season 2 anomaly, it was 5.73 ± 6.76/100 m^2 (A, G, and E until E dropped to zero). The hot spots are relatively close to centers of activity, such as natural village dwellings and areas where human activity, buffalo convergence zones (in their daily patterns of leaving the villages to forage and returning home in the evening), and suitable snail habitat occurs. There is a strong correlation between hot spots and the short distance from active sites mentioned above (r = > 0.80; Davis et al., 2002a). These hot spots are not large, averaging about 30,000 m^2 each (N = 9; Davis et al., 2002a). What is important in hot spots is not the density of snails, but a site generally favorable for snails each year where sufficient buffaloes defecate regularly, depositing thousands of *S. japonicum* eggs, so that numbers of snails become infected relatively frequently. Given the right circumstances, even a marginal snail habitat can be a hot spot.

Density of Infected Snails vs. Prevalence

It is clear that the most important indicator of risk of infection is how many snails are infected per area, not how many snails are infected per 100 or 1,000 snails, that is, prevalence. Prevalence of infected snails is not correlated with density of snails (R^2 = 0.16 in Jishan, 0.013 in Hexi). Prevalence can vary wildly in relationship to density of infected snails as seen in Fig. 23. Over 75% of snails infected per 1,000 snails occur where the density of infected snails is ≤ 6 snails per 100 m^2; 55% of the snails infected per 1,000 snails occur where the density of infected snails is ≤ 3 infected snails per 100 m^2. The spread of the number of infected snails per 1,000 snails is considerable, ranging from < 1 to 23 where the density of infected snails was one per 100 m^2. The range was three to 47 at 6 infected snails per 100 m^2.

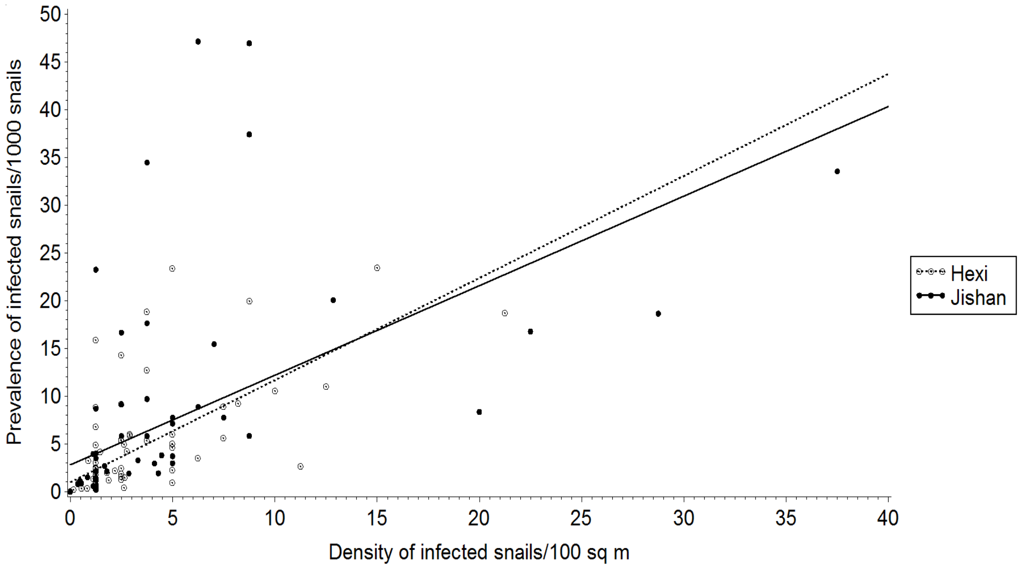

FIG. 23. Plot of density of infected snails x prevalence of infected snails for Hexi and Jishan Villages.

Probable Reasons for Intervention Failure

There are two probable reasons why snail infection rates were not decreased by the mid or end of the experiment. First, buffaloes were examined and treated only once a year. During the post treatment year buffaloes could be reinfected as infections are possible in both pre- and post-flood periods as long as the temperature does not go to 10°C or below, causing the snails to become inactive. Second, pregnant females were not treated. As the majority of the buffaloes herd was female and of reproductive age and included pregnant individuals, there was no complete control of the herd for eliminating infections. It only takes one or two infected buffalo defecating in hot spots to maintain the steady state density of infected snails over the nine seasons. Buffaloes fecal patties are immense and can contain thousands of eggs. Hot spots, as discussed above, are areas that are close to human habitation, where there is maximal and daily visitation by man and buffaloes in an environment continuously supporting snails, even if the snail density is low. Other animals can be discounted as a source of infection. Pigs would be the most likely candidates, but they were always penned. There were no dogs, goats or sheep.

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APPENDIX 1

Total snail data for two villages. *Arithmetic mean.

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean*	No./m^2 Mean*	No./4 m^2 Frames	Mean*	Per m^2 Mean*	No. of Snails
Hexi Village								
Nov-98	159	10,011	62.96	15.74	12	0.075	0.019	0.0012
May-99	120	1,677	13.98	3.5	7	0.058	0.015	0.0042
Nov-99	140	8,676	61.97	15.49	3	0.021	0.005	0.0003
Apr-00	200	6,582	32.91	8.23	9	0.045	0.011	0.0014
Dec-00	200	10,797	53.99	13.5	20	0.1	0.025	0.0019
Apr-01	200	8,641	43.21	10.8	40	0.2	0.05	0.0046
Nov-01	200	2,174	10.87	2.72	7	0.035	0.009	0.0032
Apr-02	180	3,496	19.42	4.86	21	0.117	0.029	0.006
Nov-02	200	10,631	53.16	13.29	20	0.1	0.025	0.0019
Jishan Village								
Nov-98	119	9,520	80.00	20.0	6	0.05	0.013	0.0006
May-99	140	3,585	25.61	6.4	72	0.514	0.129	0.0201
Nov-99	140	4,692	33.51	8.38	10	0.071	0.018	0.0021
Apr-00	140	3,432	24.51	6.13	3	0.021	0.005	0.0009
Dec-00	140	6,545	46.75	11.69	25	0.179	0.045	0.0038
Apr-01	140	7,800	55.71	13.93	23	0.164	0.041	0.0029
Nov-01	140	2,477	17.69	4.42	2	0.014	0.004	0.0008
Apr-02	120	2,653	22.11	5.53	4	0.033	0.008	0.0015
Nov-02	140	8,440	60.29	15.07	16	0.114	0.029	0.0019

APPENDIX 2

Zones. All snail data.

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean/ Frame	Mean/ m^2	Total No. of Snails	Mean/ Frames	Mean/ m^2	Prevalence Infected Snails
Hexi Village								
Shamo Zone								
Nov-98	39	8,350	214.1	53.53	11	0.282	0.071	0.0013
May-99	20	1,179	58.95	14.74	0	0	0	0
Nov-99	40	5,653	141.3	35.33	1	0.025	0.006	0.0002
Apr-00	60	3,639	60.65	15.16	1	0.017	0.004	0.0003

(continues)

(continued)

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean/ Frame	Mean/ m^2	Total No. of Snails	Mean/ Frames	Mean/ m^2	Prevalence Infected Snails
Dec-00	60	6,455	107.58	26.9	4	0.067	0.017	0.0006
Apr-01	60	2,215	36.92	9.23	4	0.067	0.017	0.0018
Nov-01	60	1,076	17.93	4.48	1	0.017	0.004	0.0009
Apr-02	60	1,153	19.22	4.81	1	0.017	0.004	0.0009
Nov-02	60	3,253	54.22	13.56	0	0	0	0
Houshan Zone								
Nov-98	120	1,661	13.48	3.46	1	0.008	0.002	0.0006
May-99	100	498	4.98	1.25	7	0.07	0.018	0.0141
Nov-99	100	3,023	30.23	7.56	2	0.02	0.005	0.0007
Apr-00	140	2,943	21.02	5.26	8	0.057	0.014	0.0027
Dec-00	140	4,342	31.01	7.75	16	0.114	0.029	0.0037
Apr-01	140	6,426	45.9	11.48	36	0.257	0.064	0.0056
Nov-01	140	1,098	7.84	1.96	6	0.043	0.011	0.0055
Apr-02	140	2,343	19.53	4.88	20	0.167	0.042	0.0085
Nov-02	140	7,378	52.7	13.18	20	0.143	0.036	0.0027
Jishan Village								
Village Zone								
Nov-98	40	1,128	28.2	7.05	4	0.1	0.025	0.0035
May-99	60	1,383	23.05	5.76	30	0.05	0.125	0.0217
Nov-99	60	1,193	19.88	4.97	5	0.083	0.021	0.0042
Apr-00	60	784	13.07	3.27	1	0.017	0.004	0.0013
Dec-00	60	1,757	29.28	7.32	23	0.383	0.096	0.0131
Apr-01	60	2,517	41.95	10.49	23	0.383	0.096	0.0091
Nov-01	60	324	5.4	1.35	2	0.033	0.008	0.0062
Apr-02	60	717	11.95	2.99	4	0.067	0.017	0.0056
Nov-02	60	2,093	34.88	8.72	15	0.25	0.063	0.0072
Southern Zone								
Nov-98	40	7,692	192.3	48.08	1	0.025	0.006	0.0001
May-99	40	2,094	52.35	13.09	37	0.925	0.231	0.0177
Nov-99	40	2,864	71.6	17.9	0	0	0	0
Apr-00	40	2,311	57.78	14.45	0	0	0	0
Dec-00	40	3,855	96.38	24.1	0	0	0	0
Apr-01	40	3,239	80.98	20.25	0	0	0	0
Nov-01	40	1,504	37.6	9.4	0	0	0	0
Apr-02	40	1,724	43.1	10.78	0	0	0	0
Nov-02	40	4,909	122.7	30.68	1	0.025	0.006	0.0002
Western Zone								
Nov-98	39	700	17.95	4.49	1	0.026	0.007	0.0014
May-99	40	108	2.7	0.68	5	0.125	0.031	0.0463
Nov-99	40	635	15.88	3.97	5	0.125	0.031	0.0079
Apr-00	40	337	8.43	2.11	2	0.05	0.013	0.0059
Dec-00	40	933	23.33	5.83	2	0.05	0.013	0.0021
Apr-01	40	2,044	51.1	12.78	0	0	0	0
Nov-01	40	649	16.23	4.06	0	0	0	0
Apr-02	20	649	16.23	4.06	0	0	0	0
Nov-02	20	212	10.6	2.65	0	0	0	0